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Transfer Rate Edited Experiment for the Selective Detection of Chemical Exchange via Saturation Transfer (TRE-CEST)

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Abstract

Chemical Exchange Saturation Transfer (CEST) magnetic resonance experiments have become valuable tools in magnetic resonance for the detection of low concentration solutes with far greater sensitivity than direct detection methods. Accurate measures of rates of chemical exchange provided by CEST are of particular interest to biomedical imaging communities where variations in chemical exchange can be related to subtle variations in biomarker concentration, temperature and pH within tissue using MRI. Despite their name, however, traditional CEST methods are not truly selective for chemical exchange and instead detect all forms of magnetization transfer including through-space NOE. This ambiguity crowds CEST spectra and greatly complicates subsequent data analysis. We have developed a Transfer Rate Edited CEST experiment (TRE-CEST) that uses two different types of solute labeling in order to selectively amplify signals of rapidly exchanging proton species while simultaneously suppressing ‘slower’ NOE-dominated magnetization transfer processes. This approach is demonstrated in the context of both NMR and MRI, where it is used to detect the labile amide protons of proteins undergoing chemical exchange (at rates $\sim 30 \text{ s}^{-1}$) while simultaneously eliminating signals originating from slower ($\sim 5 \text{ s}^{-1}$) NOE-mediated magnetization transfer processes. TRE-CEST greatly expands the utility of CEST experiments in complex systems, and *in-vivo*, in particular, where it is expected to improve the quantification of chemical exchange and magnetization transfer rates while enabling new forms of imaging contrast.

Graphical Abstract

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Introduction

The chemical exchange saturation transfer (CEST) family of magnetic resonance pulse sequences tag protons of a solute molecule with a non-equilibrium nuclear spin magnetization and then detects the subsequent transfer of the labeled magnetization to the bulk water signal [1]. Because the solvent water is typically far more concentrated than any solute, multiple labeling and magnetization transfer events can be cumulatively stored in the water proton pool prior to detection, thereby greatly amplifying the signal of low concentration solute protons. Accurate quantification of magnetization transfer phenomena from CEST data can then be used to probe diverse chemical properties including molecular structure, dynamics, pH, solute concentration gradients, and temperature [2–5].

The precise nature of the magnetization labeling used in a CEST experiment can take on diverse forms including continuous wave (CW) saturation [1], frequency selective excitation dephasing [2], rotational flip angle difference [6], or frequency labeling [7]. Regardless of the solute labeling method however, CEST experiments utilize a common detection strategy; solute signals are resolved via the labeling-dependent perturbations they induce in the water signal intensity following magnetization transfer. Two physical processes typically mediate the transfer of the labeled magnetization from solutes of interest to bulk water: chemical exchange, where labeled protons physically swap binding partners with the solvent water proton pool, and the through-space NOE interaction where nuclei exchange magnetization via fluctuating dipolar couplings [8].

In many biological samples containing proteins, the intermolecular through space coupling between water and internal aliphatic or olefinic protons may be very small. In these situations magnetization transfer from non-labile protons is dominated by NOE-relayed CEST effects whereby magnetization is first transferred via intramolecular NOE to labile proton species which then in turn exchange with water [9–11]. We will refer to all signals from non-exchangeable protons detected in CEST experiments as NOE-mediated signals so as to avoid making implicit assumptions about the underlying magnetization transfer process in diverse samples, and because the through-space NOE transfer step is generally the rate-limiting step governing the apparent transfer processes. Additionally, saturation transfer between water and semisolids where solutes have limited rotational freedom is commonly referred to using the specific name, MT-effect, despite the fact the same underlying transfer mechanisms are ultimately giving rise to these signals. MT-effect signals are extremely broad, owing to limited rotational averaging that can occur in semisolids.

CEST signals originating from genuine chemical exchange have typically been of greatest interest to the biomedical imaging communities, but signals from NOE transfer have been isolated from biological tissue spectra and could be used to generate diverse new types of imaging contrast [9]. Unfortunately the presence of slower transferring NOE and MT-effect

signals commonly complicates the subsequent analysis of CEST data and contaminates the spectral baseline, especially upfield of the water resonance, and often can hamper accurate data extraction [2].

Here, we describe a Transfer Rate Edited CEST (TRE-CEST) experiment that can be selectively tuned to suppress signals arising from experimentally defined ‘slower’-rates of magnetization transfer while greatly amplifying signals of more rapidly exchanging proton species. The TRE-CEST experiment begins with a variable number of identical, discrete Label Transfer Modules (LTMs) that tag solute protons prior to detection of the water signal. The high concentration of water protons (~110 M) coupled with the water’s relatively slow R_1 relaxation rate allows multiple LTMs to cumulatively store a saturation label in the water proton pool prior to detection, thereby greatly amplifying the signal effect. A frequency-resolved Z-spectrum is then constructed from a series of independent experiments where the response of the water signal is measured as a function of the LTM’s labeling frequency offset.

TRE-CEST accomplishes this transfer rate editing via the interplay between two different magnetization-labeling methods per LTM: A water band-stopped excitation pulse (excitation labeling) and, a frequency selective continuous wave (CW) labeling (saturation labeling). The initial excitation pulse is designed to rapidly equalize the Zeeman energy spin states of all the exchanging proton species in the sample simultaneously by creating single quantum coherence. After all the protons in the sample have been tagged by the excitation labeling step, the longer-duration, frequency encoding CW pulse is then selectively applied to the proton species of interest in order to continuously replace its label as it is transferred to bulk water or lost via other spin relaxation mechanisms. The duration of this continuous wave labeling in consecutive LTMs affects the signals differently depending upon their rate of magnetization transfer with water, and it is this dependence that is exploited by TRE-CEST to selectively suppress signals, and quantitate transfer rates.

Theory

A complete description of the TRE-CEST experiment requires solving a multi-pool model of the Bloch equations, but a few simplifying assumptions can be used to help form an intuitive framework for describing the experiment. To build this qualitative description of TRE-CEST we will assume that, 1) the spin-lattice relaxation of labeled protons is negligible during the course of a single LTM, 2) the water proton pool is large enough compared to labile solute proton species so that there is no back transfer of already labeled solvent protons to the solute, 3) steady state conditions are not reached over the time course of the model (an implicit requirement for item 2), and 4) labeling pulses have ideal labeling efficiency. With these assumptions in place, the TRE-CEST experiment can be explained via simple uncorrelated equations that relate the amount of signal generated in a TRE-CEST experiment to the magnetization transfer rate.

Each TRE-CEST LTM contains two different types of spin magnetization labeling, a single excitation labeling pulse followed by a longer duration CW saturation labeling pulse (Figure 1). Each of these labeling modalities contributes an amount of signal S_{EXT} and $S_{CW,i}$

respectively, from the exchanging proton species to the solvent water proton pool. Phenomenologically, the signals S_{EXT} and $S_{CW,i}$ are the changes in the Z-component magnetization of the water resonance that are caused by saturated spin magnetization from labeled solute protons. We will first consider the mathematic descriptions of the signal generated by each of these labeling modalities individually before considering how they work together in the context of a TRE-CEST experiment to selectively suppress signals in a saturation transfer Z-spectra originating from ‘slow’ rates of magnetization transfer.

Excitation Labeling Pulse

The binomial composite pulse at the beginning of each LTM is designed to simultaneously excite all the non-water protons present in the sample while maintaining alignment of the water magnetization with the Z-axis. In principle any band-stop excitation pulse shape could be used in this capacity, but we have chosen the binomial P1331 pulse element because of its relatively short duration (~1 ms) and ease of setup [12]. Although the timing diagram of the P1331 pulse may, on first inspection, have superficial similarity to the frequency labeling pair of pulses used in the FLEX experiment, it should be emphasized that the P1331 is simply a composite excitation pulse with a frequency band-stop region centered at the water resonance, and is entirely different from the frequency labeling element found in a FLEX experiment [7], which phase encodes chemical shift information into the CEST signal.

Excitation labeling in TRE-CEST rapidly labels proton species at many different chemical shift offsets relative to water simultaneously. The label imparted by the saturation pulse will not be replenished following exchange, thereby allowing the excitation pulse to start the experiment’s internal reference clock and making it possible to measure exchange rates with TRE-CEST. It should be emphasized that because the band-stopped excitation pulse labels protons at many frequencies simultaneously, they encode no frequency, and cannot on its own be used to construct a Z-spectrum. Instead, this pulse is better understood as a ‘pre-saturation’ pulse element that prepares solute protons prior to the start of the frequency-selective CW irradiation period that will ultimately be used to create the frequency response Z-spectra as is done in a typical CEST experiment. Furthermore, this water editing excitation pulse is always applied at the same transmitter offset, so the amount of saturation S_{EXT} generated by this pulse can be treated as a constant value that is added to each data point in subsequent Z-spectra. The effect of this excitation pulse is thus easily removed from the resultant TRE-CEST Z-spectra by simply subtracting this same constant offset from the spectra in order to return the baseline to zero.

The size of this constant signal offset S_{EXT} generated by the excitation pulse is determined by the relationship

$$S_{EXT} = \sum_j [x_j] \cdot \left(1 - e^{-(t_{sat} \cdot k_{MT,j})}\right) \quad (1)$$

where $[x_j]$ is the concentration of proton species j , and the final term in parentheses describes the efficiency of the magnetization transfer from the solute species j to water during time t_{sat} [2]. The summation over all j protons in the sample reflects the fact that the

label imparted by the excitation pulse comes from all the proton species present in the sample. Because the excitation pulse imparts the same amount of signal to water for every frequency offset, it has no influence on the shape of the resulting Z spectrum except for a scaling in proportion to the excitation profile. Excitation labeling in the context of TRE-CEST simply sets up the spin magnetization of the system prior to the start of the frequency encoding CW saturation pulse.

The CW Saturation Pulse

Unlike the excitation labeling discussed above, CW saturation labeling ideally produces signal from a single proton species i at the selected frequency offset in the Z-spectra. Under pre-steady-state conditions where the CW nutation frequency is much greater than $k_{MT,i}$, the rate of magnetization transfer, the signal generated by CW labeling of proton species i , $S_{CW,i}$, will scale linearly with the rate of magnetization transfer, the duration of the saturation pulse, and the sample concentration.

$$S_{CW,i} = [x_i] \cdot k_{MT,i} \cdot t_{sat}, \quad (2)$$

where the factor $[x_i]$ is the molar concentration of proton species i , and t_{sat} is the duration of the CW labeling pulse. Unlike excitation labeling, the signal produced by saturation labeling will grow linearly with the rate of magnetization transfer and saturation time, and is not bounded by the concentration of the exchanging proton species.

TRE-CEST Label Transfer Module

The amount of signal (S_i) produced in a TRE-CEST LTM can, to a first approximation, be thought of as the sum of the signals produced by the excitation pulse S_{EXT} and CW saturation pulse $S_{CW,i}$ independently. In order to understand the transfer rate editing properties of the TRE-CEST experiment however, minor deviations from this simplistic model induced by the interplay between these two pulses must be considered. In TRE-CEST, the CW saturation labeling of proton species i begins immediately following the excitation labeling pulse. While the majority of the protons tagged by the excitation label will have the full duration t_{sat} to transfer their label to water (Eq. (1)), proton species i will immediately come under the influence of the saturation pulse. In this situation, the frequency selective CW labeling element can be thought of as ‘overwriting’ the ‘excitation’ label of proton i before magnetization transfer can take place thereby negating the effect of excitation labeling on this species. Taking this interplay between pulses into account, the total signal generated by a TRE-CEST label transfer module applied to proton species i , S_i is better expressed as

$$S_i \approx \sum_j S_{EXT,j} \cdot (1 - \delta_{j,i}) + S_{CW,i}, \quad (3)$$

where $\delta_{j,i}$ is the Kronecker delta function ensuring that the $S_{EXT,j}$ factor drops to zero for the $j=i$ proton species. To summarize this in words, the total signal generated by a TRE-CEST LTM is simply the CW saturation signal $S_{CW,i}$ produced by proton i , added to one excitation labeling equivalent of all the proton species in the sample $j \neq i$.

Transfer Rate Editing

The product $t_{sat} \cdot k_{MT,j}$ appears in both Eqs. (1) and (2) and plays a special role in determining the response of the TRE-CEST experiment to proton species with different rates of magnetization transfer. If this product becomes very large, either owing to a rapid rate of magnetization transfer (large $k_{MT,j}$), or the use of a very long duration saturation pulse t_{sat} , the TRE-CEST signal will be dominated by the CW labeling component; *i.e.* when $[x_i] \cdot t_{sat} \cdot k_{MT,j} \gg S_{EXT}$, Eq. (3) can be approximated as

$$S_i \approx [x_i] \cdot k_{MT,i} \cdot t_{sat}, \quad (4)$$

and the TRE-CEST experiment on proton i is equivalent to the traditional CW saturation transfer experiment.

If on the other hand $k_{MT,i} \cdot t_{sat}$ becomes very small, the signal from species i effectively disappears, and Eq. (3) in these situations reduces to

$$S_i \approx \sum_j S_{EXT,j}, \quad (5)$$

where the excitation signal of all the other proton species in the sample dominate. By adjusting the duration t_{sat} , the experiment can be effectively tuned to situations under which Eq. (4) or (5) will hold for a given rate of magnetization transfer. It should now be noted that the signal in Eq. (5) is equivalent to the constant frequency independent offset described in Eq. (1), and that under conditions where $k_{MT,i} \cdot t_{sat}$ is small, TRE-CEST generates no additional signal on top of the experimentally determined spectral baseline. Therefore, these signals will effectively disappear from the spectra.

Adjusting the duration of the labeling period t_{sat} , will change the rate editing properties of the experiment but this may be to the detriment of the amount of signal amplification that is achieved for faster exchanging protons. To keep the signal amplification generated by TRE-CEST relatively constant as a function of the t_{sat} duration, a constant total CW labeling time T can be broken up into N sequential LTMs of duration t_{sat} each, which are applied prior to detection. The net signal $S_{i,Net}$ produced by TRE-CEST from proton i after N LTMs is thus given by

$$S_{i,Net} \approx \sum_{n=1}^N S_{i,n} \cdot e^{-[(N-n)/N] \cdot R_1 \cdot T}, \quad (6)$$

where R_1 is the spin-lattice relaxation rate of water and $S_{i,n}$ is the signal generated by the n^{th} module as defined in Eq. (3). The form of Eq. (6) is the same for all pulsed labeling methods and was originally derived for the case of the FLEX pulse sequence.

Bloch Simulations

To better characterize the rate editing properties of TRE-CEST, complete Bloch simulations were performed on a two-spin (solvent/solute) system in order to extract the predicted signal

$S_{i,Net}$ as a function of the rate of magnetization transfer (Figure 2a). The saturation signals produced by CEST experiments are here reported in terms of a Magnetization Transfer Ratio (MTR) describing the amount of saturation produced as a fraction of the total size of the water proton signal (S_0), $MTR = 1 - S_{i,NET}/S_0$. It should be noted that while for slowly exchanging protons, TRE-CEST signals grow more slowly with k_{MT} than CW-CEST (see Figure 2c). For protons with higher rates of exchange the two signals grow at the same rate in both cases, and their relative offsets no longer change. The relationship between signal growth in TRE-CEST and CW-CEST is made more obvious in Figure 2b, where the difference in MTR between TRE-CEST and CW-CEST are plotted (labeled ΔMTR), and the relative change in signal between TRE-CEST and CW-CEST has a large slope for slow rates of magnetization transfer (left side of graph), but becomes constant (a flat line) at higher rates of k_{MT} .

While TRE-CEST grows at the same rate as CW-CEST for fast rates of k_{MT} where the inequality $[x_i] \cdot t_{sat} \cdot k_{MT,j} > S_{EXT}$, holds, it is also apparent from Figure 2c that TRE-CEST will never catch up with CW-CEST for arbitrarily rapid rates of k_{MT} . This is because the signal measured in saturation transfer experiments can only be described relative to the spectral baseline, and in TRE-CEST this baseline changes as a function of N . To correct for this offset, we must subtract the factor

$$S_{baseline} = \sum_{n=1}^N \left(\sum_i S_{EXT,i} \right) \cdot e^{-[(N-n)/N] \cdot R_1 \cdot T} \quad (7)$$

from the resulting signal. This factor describes the S_{EXT} component (Eq. (1)) from every proton species in the sample as the sole signal contribution to Eq. (6). Owing to the summation over all LTMs, $S_{baseline}$ grows with N . This is exactly the effect we observe in the Bloch simulations (Figure 2b) where the difference in MTR generated by TRE-CEST and CW-CEST for rapidly exchanging protons is linearly related to the number of applied LTMs in the sequence; it should be noted that the signal loss of TRE-CEST compared to CW-CEST at the highest exchange rates in Figure 2b is, within rounding error, $(\sum_i S_{EXT,i}) \cdot N$ as predicted by Eq. (7) in situations where T_1 relaxation is negligible.

This linear signal attenuation for rapidly exchanging protons affords the experimenter nonlinear suppression of more slowly exchanging protons. This effect is well illustrated by the slopes at the left hand side of Figure 2b and in Figure 2c showing the same Bloch simulation from part a for k_{MT} rates between 0 and 5 s⁻¹. Optimizing TRE-CEST to suppress a given signal involves selecting the number of cycles N that will maximize the difference between the signal loss experienced by the undesirable signal from slow exchanges, compared to signal loss experienced by the proton species of interest with a different, faster rate of magnetization transfer.

The approximate behavior of TRE-CEST derived from Eqs. (1–7) is plotted for k_{MT} rates between 0 and 5 s⁻¹ in Figure 2d. Comparison of Figures 2c and 2d shows excellent agreement between the approximate expressions and exact simulations, in this range.

Non-ideal excitation pulse

Although care is taken to minimize the influence that the selective excitation pulse has on water proton spins, the water resonances, owing to their size, and broad Lorentzian tails could absorb a significant amount of RF labeling even from ideal pulse shapes. The direct excitation labeling of water prior to magnetization transfer of intentionally labeled proton species, compresses the dynamic range of the water signal. Because the excitation profile of the P1331 excitation pulse is not changed during the experiments, it imparts the same amount of direct saturation to water for every data point, hence these effects are generally easily removed from the spectra during post processing. Scaling this data appropriately to calculate MTR requires the collection of a reference scan for measuring S_0 in the absence of any labeling pulses.

The incomplete excitation of the non-water protons will lead to non-ideal suppression of signals arising from slowly-exchanging groups. The P1331 composite pulse provides a nice compromise between pulse duration and selectivity, but any other band-stop excitation scheme could in principle be used. The impact of the inter-pulse delay t_d on the excitation profile is explored in Supplementary Figure 1.

Methods

Sample Preparation

The bovine serum albumin (BSA) sample was prepared by dissolving lyophilized BSA (Sigma) in PBS buffer. pH was adjusted to 7.4 using dilute HCl. The solution was gently centrifuged to remove any remaining particulate matter before loading into the NMR tube or 50 mL conical. Phantoms from the MRI were prepared by placing a tightly sealed 50mL conical of the BSA solution in a two-liter container filled with tap water and centered in the container using empty falcon tubes as spacers. Egg whites from a fresh hen egg were transferred to a 5mm NMR tube and placed in boiling water until completely opaque to allow for denaturation (~10 minutes). The gelatin sample was prepared using dry porcine skin gelatin (Sigma) in PBS buffer. The resulting gelatin slurry was heated to 65°C with slow mixing until all the solid gelatin was dissolved. The sample was then allowed to cool back to room temperature prior to use.

Data collection

NMR studies were performed on a 9.4T vertical wide bore imaging system at room temperature. The water signal following labeling was quantified using a gradient recalled spin echo readout. The readout gradient is used to prevent radiation damping on the high Q-factor NMR probe. The echoes were Fourier transformed in magnitude mode and the obtained one-dimensional projections of the sample volume were numerically integrated to quantify the net water signal for each labeling offset.

Imaging studies were performed on a 7.0T whole body MRI system, where the spin echo readout element was replaced by a single-shot gradient recalled echo imaging sequence performed after each labeling offset. A 51 point WASSR dataset was collected spanning $\omega = \pm 20$ Hz along with a CEST spectral series to account for B_0 inhomogeneities across the

sample [13, 14]. Spline interpolation was then performed on each pixel in the CEST series to resample the data at the proper frequency offsets provided by the WASSR B_0 map.

Lorentzian Difference Method

A high concentration of water protons relative to solute CEST signals results in significant partial direct saturation of the water peak even at saturation offsets well removed from the spectral center. This direct saturation of water results in broad spectral shoulders in the CEST Z-spectra that complicate data analysis. To remove the effects of direct water saturation from CEST spectra, the Lorentzian difference method [9, 15] was used, where the Z-spectrum water resonance was fit to a single Lorentzian line shape in the least squares sense, and the resulting model of the water resonance was then subtracted from the experimental data to minimize baseline spectral distortions.

Asymmetry analysis

An alternative method for removing the water baseline is asymmetry analysis, where the difference is taken between the MTR measured at one labeling offset and the MTR at a symmetric point on the opposite side of the water resonance where no CEST signal from saturation transfer is presumed to exist. Expressed mathematically, the quantity $MTR_{asm}(\Delta; \omega) = MTR(-\omega) - MTR(\omega)$ is calculated for each frequency labeling offset in the spectra for $\omega > 0$ Hz. The assumption that $MTR(-\omega)$ contains no CEST signal is generally invalid and the large NOE-mediated signals present upfield of water in biological samples lead to distortions in the CEST signal that can stymie accurate quantification using this method. TRE-CEST is shown to help alleviate these unwanted effects, thereby greatly improving the accuracy of this technique.

Results and Discussion

To be resolvable via saturation transfer experiments, magnetization transfer processes must satisfy the slow-to-intermediate exchange condition of the NMR timescale ($k_{MT,i} \lesssim |\omega_i|$); this requirement places an upper limit on magnetization transfer based upon knowledge of a proton's resonance frequency alone [8]. Protons with a large upfield chemical shift are likely strongly shielded by shared electron density that raises the activation energy barrier for chemical exchange, and these protons do not typically undergo chemical exchange under physiologically relevant conditions. Magnetization transfer signals observed in such upfield resonances in biological samples are thus more likely to originate from NOE-mediated transfer processes.

In saturation transfer spectra of biological samples, one typically observes three identifiable features aside from the water signal; a chemical exchange dominated peak at around 3.5 ppm downfield of water, henceforth referred to as the APT (amide proton transfer) [16, 17] peak, a broader NOE-mediated transfer peak, spanning a region from -2 to -4 ppm upfield of the water resonance, that we will refer to as the NOE peak, and in semi-solid samples, some very broad resonances due to residual dipolar coupling-mediated MT-effects. Techniques like TRE-CEST are ultimately blind to the underlying mechanism of

magnetization transfer and are responsive only to the macroscopic rates of transfer that give rise to different spectral features.

As MT-effects in many samples are mediated by extremely rapid rates of magnetization transfer, TRE-CEST would not be expected to effectively attenuate these signals (see Supplementary Figure 3). We have chosen to investigate samples with limited or symmetric MT-effects in this work in order to focus on the validation of the TRE-CEST pulse.

Both the APT and NOE ‘peaks’ are better described as spectral envelopes that are comprised of the resonances of many different proton species with similar chemical shifts. This overlap is generally due to three factors, the significant line broadening of protons undergoing rapid magnetization transfer, the small chemical shift differences among those protons, and the finite bandwidth of the saturation labeling pulse. Because these underlying signals are typically unresolvable in CEST spectra of complex samples, we will treat them as single resonances in the following analysis while keeping their potentially diverse underlying origins in mind in situations where this simplistic treatment makes understanding the experiment more difficult.

The rate-limiting step of NOE-mediated transfer is determined by the distance between the atomic nuclei and the global rotational correlation time of the labeled proton species. The APT envelope on the other hand has contributions from proton species that can be dominated by NOE or chemical exchange mediated transfer mechanisms. As the rates of chemical exchange are largely dependent upon the unique chemical environment of each proton species, they can vary greatly amongst protons in the same molecule. Thus the APT envelope has a much greater diversity in rates of k_{MT} that can span from 0 to the fast exchange limit on the NMR timescale ($\sim 1,200 \text{ s}^{-1}$ for the protons and static B_0 field strengths considered in the following sections).

The APT peak is typically of greatest experimental interest because it is a generally sharper spectral feature than the NOE envelope, and because chemical exchange is a more responsive probe of the underlying chemical properties of the sample, including temperature and pH. The presence of the NOE peak on the opposite side of the water resonance however poses significant problems for subsequent efforts to quantify the data using a model independent asymmetry analysis method. By an unfortunate coincidence, significant NOE peaks in CEST spectra occur at a roughly symmetric point about the water line with respect to the APT peak in biological samples. This inherent symmetry causes serve baseline distortions surrounding the peak of interests following asymmetry analysis and prevents accurate quantification using this model independent analysis method. It is possible to work around this limitation by fitting the full Z-spectra to a multicomponent model of saturation transfer in a given chemical system, but due to the spectral overlap the resulting data are typically highly model dependent and may not work for complex samples.

The unique ability of TRE-CEST to suppress saturation transfer signals undergoing slower rates of magnetization transfer can be leveraged in these situations to selectively remove slowly exchanging components of the NOE peak upfield of water while preserving the more rapid chemical exchange component of the APT peak downfield of the water line, thereby

making accurate asymmetry analysis practical in many situations. To demonstrate the ability of TRE-CEST to clean up the spectral artifacts arising from asymmetry analysis in CEST data, we first performed the experiment on a sample of semi-solid heat-denatured egg white, which has significant APT and NOE spectral features. As demonstrated by the black line in Figure 3, the NOE signal generates a large baseline roll surrounding the APT peak following asymmetry analysis. This baseline distortion creates areas of physically impossible negative magnetization transfer ratios surrounding the CEST peak that cannot be removed by any physically based model of the system, and that also distort the apparent shape and amplitude of the APT peak. When TRE-CEST is used to selectively suppress signals from the NOE peak, this baseline roll is completely removed (see red line Figure 3) thus greatly improving the quantification potential of model independent CEST experiments both in terms of signal amplitude and line shape analysis, without the significant loss in signal intensity that would come about from using spectral difference techniques [6].

The quantitative potential of TRE-CEST is further enhanced by the dependency of the TRE-CEST signal S_i on t_{sat} and the rate of magnetization transfer k_{MT} described in the theory section. To better quantify this relationship, a Bloch-McConnell equation solver was implemented in MATLAB to simulate TRE-CEST and CW-CEST experiments on theoretical systems. To explore the experiment's unique dependence on the product $k_{MT,i} \cdot t_{sat}$, the duration t_{sat} was varied in a number of successive simulations while keeping the net CW labeling time, T_{total} , of the experiments equal to the labeling time of a control CW CEST simulation. The control CW CEST experiment serves as a constant reference point for describing rate selective signal attenuation of a given TRE-CEST experiment. The duration of CW labeling during each TRE-CEST LTM, t_{sat} , is set as a function of the number of TRE-CEST LTMs in the experiment such that $t_{sat}(N) = T_{total}/N$, thereby reformulating N as a new independent variable describing the duration t_{sat} . By comparing the relative size of the predicted TRE-CEST signal to that of an equal duration, T_{total} , CW labeling experiment, one can describe the transfer rate attenuation of TRE-CEST as function of N compared to a fixed reference point. Doing this for a number of rates of magnetization transfer $k_{MT,i}$, an iso-transfer contour surface is then constructed describing the effect of the product $k_{MT,i} \cdot t_{sat}$ on TRE-CEST signals relative to the comparable CW-CEST experiment performed under identical conditions.

Twelve representative iso-transfer profiles for values of k_{MT} spanning 1 s^{-1} to 200 s^{-1} are plotted as colored contour lines in Figure 4a. As expected, TRE-CEST iso-transfer profiles of the modeled protons with the fastest exchange rate show the least dependence on the duration $t_{sat}(N)$, while the iso-transfer profiles of slower transferring signals are greatly influenced by the $t_{sat}(N)$ duration and resemble exponential decay profiles. The results of these simulations were then compared to experimental TRE-CEST spectra of 12% BSA. The APT peak intensity is plotted with black diamonds and NOE peak intensity is plotted with black circles on the iso-transfer profile plot. Because we are only interested in the relative change in peak intensity and the water resonance is assumed to be unaffected by the P1331 pulse element, it is not necessary to deconvolute the water resonance to perform this transfer rate analysis, and peak intensities can be read directly from peak heights in the Z-spectra.

The data from the experimental Z-spectrum following removal of the water resonance using the Lorentzian difference method are shown in Figure 4b for comparison.

Visual inspection of the experimental data for the BSA sample shows that the experimental iso-transfer profiles are bounded between $2\text{--}5\text{ s}^{-1}$ for the aliphatic peak envelope centered at -3.5 ppm and between $20\text{--}35\text{ s}^{-1}$ for the amide peak envelope centered at 3.0 ppm . These transfer rate estimates are in close agreement with consensus literature values that have been made under similar conditions.[18] Tellingly however, the experimental iso-transfer curves show systematic deviations from the general shape predicted by Bloch simulation. The APT peak (black diamonds) in particular has a significantly flatter profile than any of the surrounding simulated iso-transfer contours. This deviation is indicative of the presence of many distinct proton species that are all undergoing unique rates of saturation transfer but that are spectroscopically unresolvable and collectively contribute to the observed APT spectral envelope. Indeed, pulsed saturation transfer methods like FLEX that are more sensitive to faster exchanging species measure average magnetization transfer rates for amide envelopes that are orders of magnitude faster than CW based saturation measurements because they selectively detect the fastest transferring components of the spectral envelope [19]. TRE-CEST encodes frequency response data via the CW labeling pulse and yields transfer rate measures that are most consistent with traditional CW-CEST, but the experimental response to the number of discrete LTMs provides additional information about overlapping signal components that may be present in the spectral envelope and that would not be simultaneously discernible via other saturation transfer approaches. Despite its equally heterogeneous origin, the NOE peak on the other hand, yields an average transfer rate via TRE-CEST that is far more consistent with a single rate of magnetization transfer. This is because the NOE transfer rate is determined by the rotational correlation time of the BSA molecule, and although the NOE envelope may be composed of protons in diverse chemical environments, they are all tumbling along with BSA, and consequently are bounded by a common upper NOE-mediated transfer rate limit (Figure 4). The uniformity and slow transfer rate of the NOE peaks makes them easier to both selectively and uniformly suppress via TRE-CEST.

When dealing with spectroscopically distinct signals, the signal decay profiles as function of N generated by TRE-CEST, can be used to quantitate transfer rates and provide a complementary, yet orthogonal, means to QUEST and QUESP experiments for the measurement of magnetization transfer rates [20]. In a clinical MRI setting these transfer profiles may ultimately be turned into a contrast mechanism that can help to distinguish between different tissue types and *in-vivo* conditions based upon the diversity and distribution of the underlying transfer rate components. To demonstrate the suitability of the TRE-CEST experiment in the context of imaging, we implemented the experiment on a 7.0T whole body MRI system, replacing the detection component shown in Figure 1a with a slice selective gradient recalled echo single-shot imaging sequence. We then acquired a Z-spectrum from a phantom containing a 12% BSA sample in a 50mL conical tube. The Z-spectra were constructed from fifty-one identical slices of the phantom, each with a different CW-labeling offset within the range $-8.5\text{ to }8.5\text{ ppm}$ (see Figure 5). Variations in the effective B_0 field in each pixel of the slice were corrected for by using the WASSR approach

[13]. While the CW-CEST experiment produces about ~7% MTR contrast in the -4 to -2 ppm spectral region of the BSA sample, which is dominated by NOE-mediated transfer (top right panel Figure 5), TRE-CEST effectively eliminates these signals (bottom right panel Figure 5), thus greatly simplifying the spectra and imaging contrast, while preserving the amide proton signals that undergo chemical exchange mediated transfer with water. TRE-CEST thus could greatly improve the quantifiability of CEST data, provide a rapid and easy means for distinguishing between NOE and chemical exchange dominated magnetization transfer, and the dependency of TRE-CEST signals on N could serve as a basis for new types of imaging contrast.

By combining a pulsed water band stopped excitation-labeling element with frequency selective CW labeling, TRE-CEST achieves comparable sensitivity to CW CEST for the fast exchanging protons, which are commonly of most interest, while simultaneously suppressing the signals of resonances undergoing slower rates of magnetization transfer and commonly hamper signal quantification. Other experiments like VDMP-CEST [18] make use of variable spacing between labeling pulses to selectively bias signals depending on their transfer rate. Another method, CERT [21], encodes transfer rate information in the flip angle that a B_1 field can impose prior to transfer, but these approaches cannot actively suppress slowly exchanging signals as is done in TRE-CEST. In contrast, the FLEX method of frequency labeling can selectively remove slowly transferring components by keeping the total evolution time T of the frequency labeling LTM short enough that magnetization transfer is unlikely to occur to a significant degree, but this approach ultimately comes at the expense of spectral resolution.

The offset labeling modality used in the TRE-CEST experiments is generalizable, and opens the possibility of replacing the CW frequency labeling pulse with a FLEX labeling module that would enable the simultaneous suppression of the effects from both very fast (owing to the FLEX T_2 filter) and very slowly exchanging groups, thus providing a potentially highly selective band-pass filter for magnetization transfer. Tighter controls on the range of transfer rates detected by such CEST experiments could also make it possible to pick isolated endogenous reporter signals from the CEST signal envelope of complex tissues and could lessen the need for exogenous CEST-based contrast agents for quantification of physiological properties. In addition to frequency labeling, TRE-CEST could also be combined with other spectral editing approaches like uniform MT (uMT) CEST [22, 23], VDMP[18], or ZAPI[24] to attenuate MT-effects present in semisolid samples (see for example Supplementary Figure 3), further simplifying *in-vivo* saturation transfer datasets and improving quantification in heterogeneous samples.

Conclusion

We describe here a method for the separation of fast from slow magnetization transfer processes based on a transfer-rate editing pulse sequence. The method is demonstrated both spectroscopically and by imaging implementations, where NOE-mediated transfer processes, which typically occur at slow rates, are shown to be separated out from the true chemical exchange signals in CEST spectra. The application of this method to imaging could provide a means for authenticating genuine CEST and NOE effects in tissues, but the

parameters in the transfer-rate editing step could also be used as a tunable contrast mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We describe a pulse sequence for selectively removing NOE signals from CEST spectra.
- The experiment selectively edits CEST signals based upon their rate of magnetization transfer with water.
- Maintains high sensitivity; amplifies signals by orders of magnitude compared to direct detection methods.
- The experiment can suppress artifacts from asymmetry analysis greatly improving the quality of CEST data.

Figure 1.

a) TRE-CEST pulse sequence with N label transfer modules (LTMs) applied prior to detection of the water signal. For MRI experiments, the direct detection is simply replaced by a gradient echo readout element. A detailed insert of the LTM used here is shown at the top. Black flags represent the placement of the transmitter on the center water frequency, while red flags denote moving the carrier to selectively saturate a given proton species. The pulse tip angle is $\alpha = 11.25^\circ$, and the inter-pulse delay is $t_d = 1/(2 \cdot \omega)$ where ω is the frequency offset of the slow exchanging resonance in Hz relative to the spectral center defined by water (see Supplementary Figure 1). The small, hatched pulse represents a low power frequency selective saturation element. Note the timing diagram is not drawn to scale and t_{sat} is typically much longer than the P1331 pulse element. (b) Bloch simulation of Z-spectra generated by TRE-CEST (red, $\gamma B_1 = 75 \text{ Hz}$, $t_d = 416 \mu\text{s}$, $n = 10$, $t_{sat} = 100 \text{ ms}$) and continuous wave saturation CEST (black, $\gamma B_1 = 75 \text{ Hz}$, $t_{sat} = 1.0 \text{ s}$). The simulation was performed on a three-spin system, with a 110 M water signal at 0 ppm. The simulated proton resonating at +3 ppm has $k_{MT} = 50 \text{ s}^{-1}$, $R_2 = 75 \text{ s}^{-1}$, and $x_s = 0.9\%$ of water. The proton at -3 ppm has $k_{MT} = 0.5 \text{ s}^{-1}$, $R_2 = 200 \text{ s}^{-1}$, and $x_s = 36\%$ of water. The baseline of the TRE-CEST spectra is offset by the factor S_{EXT} .

Figure 2.

(a) Numerical Bloch simulations of TRE-CEST (the number of cycles N is indicated on the y-axis to the right) and CEST signals generated as a function of the rate of magnetization transfer. Simulations are for a two proton pool system; $\gamma B_1 = 75 \text{ Hz}$, $R_1 = 1.0 s^{-1}$, $R_2 = 30 s^{-1}$, with a 0.5% mole fraction for the ‘solute’ proton species. The total labeling time T was 1.0 second corresponding to a t_{sat} of 1000ms, 200 ms, 100 ms, 50 ms, 3.3 ms for $N = 0, 5, 10, 20$, and 30 LTMs, respectively. The downward inflection of the curves around $k_{MT} = 100 s^{-1}$ is due to the back transfer of labeled solvent protons to the solute proton pool. (b) Difference plot between signals generated by TRE-CEST and the CW-CEST experiment (ΔMTR) derived from the Bloch equations. Note that for large rates of magnetization transfer, the TRE-CEST and CEST signals grow linearly in relation to each other. (c) Detailed zoom of part (a) highlighting magnetization transfer rates from 0 to $5 s^{-1}$. (d) Transfer function from 0 to $5 s^{-1}$ calculated using the approximate equations of S_i . The simplifying assumptions implicit in the derivation of Eqs. (1–7) that are most valid for slowly exchanging protons (Eq. 6). (see Supplementary Figure 2 for a comparison at greater values of k_{MT}).

Figure 3.

Asymmetry analysis of CEST (black) and TRE-CEST (red) Z-spectra collected on heat denatured egg white. In the CEST spectra ($t_{sat}=1.0$ s, $\gamma B_1=50.0$ Hz), the amide proton peak at 3.5 ppm is exaggerated by a large NOE-mediated signal upfield of water. The broad asymmetric signal of these protons in NOE-mediated contact with water gives rise to distortions throughout the spectra produce a physically impossible negative MTR signal at ~5 ppm that greatly complicates fitting the CEST curve to a baseline for subsequent data analysis. In contrast, TRE-CEST ($t_{sat}=25$ ms, $\gamma B_1=50.0$ Hz, $N=40$) selectively suppresses the aliphatic signals on the opposite side of the water resonance undergoing slow NOE-mediated magnetization transfer to water and eliminates the baseline artifact following asymmetry analysis.

Figure 4.

(a) Bloch-McConnell simulation of TRE-CEST signal relative to CEST. Data were plotted as a function of N , the number of LTMs, used to supply a cumulative 1.0s of CW labeling with a 75.0 Hz γB_1 field. The response of TRE-CEST to various rates of polarization transfer (colors) is simulated. It is seen that the TRE-CEST signals approach the same sensitivity as traditional CEST for the fastest exchanging protons (or the longest durations of t_{sat} $N=1$). Black diamonds and circles correspond to experimental data from part (b). The Lorentzian Difference Z-spectra of 12% (w/v) BSA collected using both CW-CEST and TRE-CEST in a 9.4T vertical bore scanner are shown in (b). The total CW saturation time was 1.0s for all spectra (for TRE-CEST $t_{sat}=1.0/n$) using a 75.0 Hz γB_1 field. The P1331 pulse was optimized to maximally excite resonances at ± 3 ppm by setting $t_d = 417 \mu s$ for this B_0 field strength.

Figure 5.

Data obtained on a 7.0T whole body MRI system using GRE planar imaging detection on a 12% BSA phantom in a 50mL conical. (top) Average Lorentzian difference Z-spectrum using CEST (black) and TRE-CEST (10 cycle, red) labeling modalities, $\gamma B_1=12.5\text{Hz}$ and a total saturation time of 1.0s. A total of 51 CW irradiation offsets spanning -8.5 to 8.5 ppm ^1H were collected in steps of 100 Hz and B_0 correction was calculated for each pixel using the WASSR [13, 14] experiment with 1 Hz data interpolation. (Bottom) CEST and

TRE-CEST images from the integrated spectral regions highlighted at the top. The dotted yellow line delineates the edge of the container and the pixels.

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